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(54) **Novel lipolytic enzymes and their use in detergent compositions.**

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EP 0 218 272 B1

Description

The present invention relates to enzymes for the enzymatic degradation of fatty materials. More specifically, this invention relates to novel lipolytic enzymes with improved lipolytic activity under washing conditions, which make them particularly suitable for use as detergent additives. The invention is also directed towards a process for preparing the novel lipolytic enzymes, their use as additives for washing compositions and to a washing process using these washing compositions. Furthermore, the invention is directed to detergent composition comprising the novel lipolytic enzymes.

The enzyme of this invention comprises at least one of the multiplicity of lipolytic enzymes produced by certain microorganisms and in particular certain bacteria, which have been found to differ one from the other in physicochemical and enzymatic properties.

A special problem associated with laundry cleaning refers to the removal of stains of a fatty nature. This problem will be aggrevated still further if the trend towards lower washing temperatures persists. At the moment, the fat containing dirt is emulsified and removed as a result of the washing process at high temperature and high alkalinity.

Due to the present trend for energy saving, there is a strong tendency toward the use of relatively low washing temperatures, i.e. around 40 °C or lower. There is therefore a need for lipases, which are effective at the lower washing temperatures, stable in high alkaline detergent solutions and stable under storing conditions in both solid and liquid detergent compositions.

Although the use of lipolytic enzymes in detergent compositions has been known for many years (see e.g. the references mentioned on page 1, lines 36-38 of British Patent Specification No. 1,442,418), they appear to be rather unsatisfactory in practice since they exhibit only a very low cleaning efficiency under washing conditions and they do not meet the present stability requirements. For a comprehensive review article, reference is made to H. Andree et al.,J. Appl. Biochem., 2 (1980) 218-229, "Lipases as Detergent Components ".

Lipolytic detergent additives are also known from, e.g., British Patent Specification No. 1,293,613 and Canadian Patent No. 835,343.

U.S. Patent No. 3,950,277 and British Patent Specification No. 1,442,418 disclose lipase enzymes combined with an activator and calcium and/or magnesium ions, respectively, which are utilized to pre-soak soiled fabrics and to remove triglyceride stains and soils from polyester or polyester/cotton fabric blends, respectively. Suitable microbial lipases for use herein (apart from animal and plant derived lipases) are said to be those derived from Pseudomonas, Aspergillus, Pneumococcus, Staphylococcus, and Staphylococcus toxins, Mycobacterium tuberculosis, Mycotorula lipolytica, and Sclerotinia.

British Patent Specification No. 1,372,034 discloses a detergent composition comprising a bacterial lipase produced by Pseudomonas stutzeri strain ATCC 19154. Furthermore, it is recommended that the preferred lipolytic enzymes should have a pH optimum between 6 and 10, and should be active in said range, preferably between 7 and 9. Around 1970, this presumed Pseudomonas stutzeri strain was reclassified as Pseudomonas aeruginosa, as appears for example from the ATCC catalogues.

European Patent Application EP-A-0130064 discloses an enzymatic detergent additive comprising a lipase isolated from Fusarium oxysporum with an alleged higher lipolytic cleaning efficiency than conventional lipases.

As a result of extensive research and experimentation lipase preparations have been surprisingly found, the active component of which is a lipolytic enzyme, which exhibits lipase activity under modern washing conditions, i.e. it is stable and effective at high detergent concentrations, at high pH and at low washing temperatures. These lipase preparations, which are preferably in the form of enzymatic detergent additives, can be obtained by cultivation of suitably selected microorganisms of Pseudomonas pseudoalcaligenes.

Accordingly, the invention provides an enzymatic detergent additive, the active component of which is a lipolytic enzyme, characterized in that the lipolytic enzyme is an enzyme obtained from a lipase producing strain selected from the group consisting of Pseudomonas pseudoalcaligenes CBS 467.85, CBS 468.85, CBS 471.85, CBS 473.85 and ATCC 29625, and mutants and variants thereof, said enzyme being further characterized by:

a) a pH optimum in the range of 8 to 10.5, measured in a pH-stat under conditions of TLU determination;

b) exhibiting effective lipolytic activity in an aqueous solution containing a detergent at a concentration up to 10 g/l of solution under washing conditions at a temperature of 60 °C or below and at a pH between 7 and 11, and preferably between about 9 and 10.5. The pH optimum is determined in a pH-stat under conditions of TLU determination, which is described hereinafter.

It is well known to people skilled in the art that strains of the species Pseudomonas pseudoalcaligenes, exemplified by the strains DSM 50188 (the same as ATCC 17440, Type strain) and DSM 50189, do not

produce lipases. However, it is also known that said species is rather heterogenous (see e.g. N.J. Palleroni, Bergey's Manual of Systematic Bacteriology, Vol. 1, N.R. Krieg, Ed., Williams and Wilkins, Baltimore/London 1984, p. 173).

N.W. Schaad et al., Int. J. Syst. Microbiol., 28 (1978) 117-125, propose the creation of a new subspecies, Pseudomonas pseudoalcaligenes subspecies citrulli, to include a group of strains that are pathogenic for watermelon. Strain ATCC 29625 is the type strain.

M. Goto, Int. J. Syst. Microbiol., 33 (1983) 539-545, discloses a bacterial disease characterized by the development of rectangular lesions on the leaves of konjac, which is caused by a subspecies of Pseudomonas pseudoalcaligenes, named konjaci. The type strain is ATCC 33996.

Although the phenotypic characteristics of the two subspecies citrulli and konjaci differ somewhat from each other and from the type strain ATCC 17440, it is considered by those skilled in the art that these subspecies belong to the species Ps. pseudoalcaligenes. Their appearance shows the natural variation found in the phenotypic characteristics of the Ps. pseudoalcaligenes species. The variation of the phenotypic characteristics is expressed, among other traits, in the production of lipase by the new subspecies citrulli and konjaci of the Ps. pseudoalcaligenes species.

The lipolytic enzymes from the lipase producing strains of Pseudomonas pseudoalcaligenes can be used as active components in detergent compositions or enzymatic detergent additives. The prior art does not teach or suggest that the lipases could be obtained from Pseudomonas pseudoalcaligenes and that they could also be used effectively in detergents.

According to another aspect of the invention, there is provided a process for producing a lipolytic enzyme suitable for use in detergents, characterized in that a strain of Pseudomonas pseudoalcaligenes is cultivated in a nutrient medium therefor to form a lipase rich broth and lipase is isolated from the broth, with the proviso that the Pseudomonas pseudoalcaligenes strain is not ATCC 29625 nor ATCC 33996.

In a preferred embodiment a lipase enzyme can be obtained by cultivating, under usual cultivating conditions, a novel isolate of Ps. pseudoalcaligenes selected from the group consisting of the strains with the internal numbers Sp 9, IN II-5, Gr VI-15 and M-1, which were deposited on August 8, 1985, with the Centraal Bureau voor Schimmelcultures (CBS) at Baarn, the Netherlands, under numbers CBS 467.85 (7181), CBS 468.85 (7182), CBS 471.85 (7185) and CBS 473.85 (7187), respectively. These isolates are identified by the phenotypic characteristics listed in Table 1. The four strains form also an aspect of the invention.

The lipases produced by each of the 4 selected strains of Pseudomonas pseudoalcaligenes, defined above, show a surprisingly good stability and effectiveness under washing conditions. The same applies to the lipase produced by the Type strain of Pseudomonas pseudoalcaligenes subspecies citrulli, indicated above, the useful properties of which could in no way be derived from the reference of Schaad et al.

The preferred lipase preparations of this invention are those which cause a hydrolysis of at least about 10% and preferably at least about 20% of the recovered fats under the conditions described in Example 9 hereinafter for powder detergent at a minimum detergent concentration of 2 g/l.

TABLE 1. Characteristics of Pseudomonas pseudoalcaligenes strains Sp 9, IN II-5, Gr VI-15 and M-1

Character \ Strain		Sp 9	IN II-5	Gr VI-15	M-1
Shape and size of cells		rods	rods	rods	rods
Polymorphism		absent	absent	absent	absent
Motility		motile	motile	motile	motile
Formation of spores		none	none	none	none
Gram staining		-	-	-	-
Oxidase reaction		+	+	+	+
Anaerobic dextrose		-	-	-	-
Aerobic:	dextrose	-	-	-	-
	maltose	-	-	-	-
	sucrose	-	-	-	-
	D-xylose	-	-	-	+
Arginine dihydrolase		+	+	-	+
Lysine decarboxylase		-	-	-	-
Ornithine decarboxylase		-	-	-	-
Urease		-	-	-	-
Gelatin hydrolysis		-	-	-	-
Catalase		+	+	+	+
Beta-galactosidase (ONPG-method)		-	-	-	-

TABLE 1 (contd)

Character \ Strain		Sp 9	IN II-5	Gr VI-15	M-1
Indole formation		-	-	-	-
Reduction of nitrate		+	+	+	+
Nitrate reduced passed nitrite (N_2-gas production)		-	-	-	-
Starch hydrolysis		-	-	-	-
Phenylalanine deamination		-	-	-	(+)
Citrate utilization		+	+	+	+
Growth on:	1% cetrimide	+	-	n.t.	n.t.
	6.5% NaCl	-	n.t.	n.t.	n.t.
	McConkey	+	+	+	+
Growth at:	4°C	-	-	-	-
	42°C	+	+	+	+
Hydrogen sulfide production		-	-	n.t.	n.t.
Behaviour towards oxygen		aerobic	aerobic	aerobic	aerobic
Nutritive requirements		none	none	none	none
Pigment formation on:					
	King-A medium	-	-	-	-
	King-B medium	-	-	-	-
Source of isolate		soil (Spain)	soil (India)	soil (Greece)	soil (Malaysia)
n.t. = not tested (+) weak or delayed					

According to another aspect of the invention there are provided washing compositions containing a lipase according to the invention together with a detergent and optionally other ingredients which are commonly used in detergent compositions. The ingredients of the detergent compositions of the invention may include, in addition to the essential lipase one or more of the following:

1. surfactants commonly used in enzymatic detergent compositions. Generally, naturally or synthetic

surface active compounds may be used, e.g. water-soluble soaps, cationic, anionic, non-ionic, ampholytic or zwitterionic surfactants. An example of a commonly used surfactant of this type is dodecyl benzene sulphonate. Generally the surfactants, which may be used alone or in an admixture, are present in amounts of about 4 to 50% w/w of the washing composition,

2. water softeners such as complex phosphates, e.g. alkali metal tripolyphosphate or an alkali metal pyrophosphate or zeolites, preferably in amounts of up to 40% by weight of the washing composition. Furthermore or alternatively compounds such as alkali metal cyano-triacetate or alkali metal citrate may be included, showing a complex action in washing compositions,

3. alkali metal silicate or weakly alkaline compounds such as alkali metal bicarbonate, usually up to 20% by weight,

4. fillers, such as alkali metal sulphate,

5. compounds, such as carboxymethylcellulose, perfumes, optical brighteners, buffering compounds, polyalkylene glycols or ethanol,

6. other types of enzymes such as proteases and amylases.

In the enzymatic washing composition according to the invention, the lipase activity is preferably in the range of from 1 to 20,000 TLU/g of composition, while the proteolytic enzyme activity is preferably in the range from 50-10,000 Delft Units/g of washing composition. One TLU (True Lipase Unit) is defined as the titratable fatty acids equivalent to the amount of 1 umole NaOH per minute (see also page 16 hereinafter). The Delft Units are defined in J. Amer. Oil Chem. Soc. 60 (1983), 1672.

Another group of compounds which can be incorporated in the washing compositions according to the invention are bleaching agents such as alkali metal perborate, especially sodium perborate, alkali metal percarbonate, such as sodium percarbonate, peracids and salts thereof, and activators for these bleaching agents such as TAED. When the washing composition contains perborates, percarbonates, activators and optical brighteners on the one hand and proteases on the other hand, it is highly surprising that the lipase preparations of the present invention continue to exhibit their high stability in the presence of these ingredients.

The washing compositions of the invention may be prepared in the usual manner, for example by mixing together the components or by the preparation of an initial premix, which is subsequently finished by mixing with the other ingredients. According to one possible preparation route, one or more lipase preparations are mixed with one or more of the other compounds to make a concentrate of a predetermined enzymatic activity, which concentrate can then be mixed with the other desired components.

According to a particularly preferred embodiment, the lipolytic enzymes of the invention are in the form of an enzymatic detergent additive. This additive may also contain one or more other enzymes, for example a protease and/or an amylase, which can be used in modern washing compositions, and one or more other components, which are commonly used in the art, for example a non-ionic, salt, stabilising agent and/or coating agent. Preferably, the enzymatic detergent additive comprises besides a lipase of the invention a protease and optionally an alpha-amylase. It has been found that proteolytic enzymes do not break down the lipolytic enzymes of the invention. The enzymatic detergent additives according to the invention are generally mixed with one or more detergents and other components known in the art to form washing compositions.

According to a specific embodiment, an enzymatic detergent additive is used, wherein the lipase activity is in the range of from 10^2 to 10^6 TLU/g of additive, while the optionally present proteolytic activity is in the range of from $5x10^4$ to 10^6 Delft Units/g.

The enzymatic detergent additives of the invention may be in the form of, for example, granulates or prills, prepared according to methods which are generally known in this specific area of the art. See e.g. British Patents 1,324,116 and 1,362,365 and U.S. Patents 3,519,570, 4,106,991 and 4,242,219.

In a specific embodiment of the invention, the enzymatic detergent additive is provided in liquid form with an enzyme stabilizer. This stabilizer is e.g. propylene glycol. Such liquid additives are preferably used in liquid detergent compositions.

The stable and effective lipase preparations of the present invention can be suitably prepared by cultivating the microorganisms defined hereinbefore under appropriate conditions. In order to obtain high yields of enzyme, media containing readily assimilable carbon and energy sources are necessary, as is a nitrogen source of organic origin such as casein. More preferably, a fat or oil is added to the culture medium as well as calcium and magnesium salts and trace elements.

According to a preferred embodiment of the invention the cultivation process is carried out in skim milk, previously diluted with water in a ratio of from 1:1 up to 1:25 w/v. Good aeration is necessary during fermentation. The pH of the medium is suitably kept between 6 and 10 and preferably between 6.5 to 9.

The invention provides further a washing process, using a detergent composition of the invention. Such

a process may be satisfactorily carried out at a temperature of about 60 °C or below and preferably at 30-40 °C, at a pH usually between 7 and 11. The washing time is generally between 10 and 60 min. More preferably for the washing process, a washing solution is used, containing the detergent composition of the present invention in an amount in the range of from 0.5 to 15 g per litre of washing solution, preferably 1-10 g/l.

Performance of lipases in the washing process

Modern detergent compositions contain ingredients chosen for optimum efficacy in the washing process. It is self-evident that if a lipase is to be used in a modern washing process, it should be compatible with the usual active and effective ingredients found in the modern detergent formulations. To this end a number of test criteria has been chosen which may be used for the demonstration of the activity and effectiveness of the lipases of the invention, under washing conditions. These criteria are:

1. Activity of lipases in the presence of a widely used detergent builder such as sodium tripolyphosphate (TPP). This criterium is significant, since it is a widely held belief that lipases are dependent on alkali metal ions such as calcium for their activity and stability. Such calcium dependent lipases would thus not be suitable for use in modern washing processes.
2. Activity of lipases in modern detergent solutions. There are numerous modern detergent compositions which may be used for testing the activity and effectiveness of alkaline lipases. We have chosen to use, among others, a powder detergent composition and a liquid formulation as typical examples of widely used modern detergent formulations. These typical modern detergent compositions are:
   - ALL® (powder), a product from Unilever, The Netherlands. In our tests we used ALL-base (obtained from Unilever Research, Vlaardingen, The Netherlands) which is the same formulation as ALL, without perborate, bleach activator (TAED) and enzymes. ALL is a Registered Trade Mark.
   - TIDE® (liquid), a product from Procter & Gamble and commercially available in the USA. For our tests, the enzymes present in this formulation were inactivated by heat treatment. TIDE is a Registered Trade Mark.
3. Activity of lipases in ALL including proteases. As proteases are an important ingredient of modern detergent compositions, the activity and effectiveness of lipases should be manifest in the presence of this detergent ingredient (together with the surfactant matrix).
4. Activity of lipases in the presence of a typical bleaching agent (such as perborate) and a bleaching activator (such as TAED). Bleaching agents (and their activators at lower washing temperatures) are important ingredients of some modern detergent compositions. Activity and effectiveness of lipases in their presence are considered a significant criterium.

In order to evaluate the contribution of the lipases of the invention to the removal of oils and fats from textiles, an appropriate test system is necessary which allows an unequivocal determination of the activity of the lipases of the invention in washing solutions.

The test-fabrics commonly used, such as EMPA 101 and EMPA 102 (commercially obtainable from Eidgenössische Materialprüfungs und Versuchsanstalt, St. Gallen, Switzerland) have the drawback that detergency is measured as removal of pigment. Whether the removal of pigment from EMPA 101 and 102 fabrics may be directly correlated to the removal of fats or fatty acids is a pertinent question and for this reason alone these fabrics are not considered to be relevant for the evaluation of lipase performance.

Other test systems, for example as described in EP-A-0130064, also rely on the removal of dye from an artificially soiled fabric. The same objection made to the use of EMPA 101 and EMPA 102 test fabrics does also apply here. Moreover, in the test system of the above-mentioned patent application, emulsified fat is used as a substrate for alkaline lipases. This type of fat does not correspond with most of the fat stains which have to be tackled by a modern washing solution, in that such stains are not in emulsified form and non-emulsified fat as such is in contact with the fabric.

The test system described by Andree et al., J. Appl. Biochem., 2 (1980) 218-229, uses radioactively labelled fat deposited on the fabric. After the wash process the radioactivity on the fabric swatch is measured and related to lipolytic activity. In this system, however, it is not possible to distinguish between the radioactivity due to fat and that due to fatty acids and therefore this test system is not a reliable measure of the performance of alkaline lipases in the washing process.

For the purpose of this invention a new test has been developed, hereinafter called SLM-test, which is used for the evaluation of alkaline lipases and their activity and effectiveness in the washing process. The SLM-test uses the same principles as the method developed by T. Hashimoto et al., Yukagaku 34 (1985), 606-612, but the time necessary for the analysis has been drastically reduced. The method includes using immobilized, non-emulsified fat or oil on a fabric as the test stain, extracting the swatch after the washing

process and analysing the extracts for fats and fatty acids. Depending on the conditions used, fatty acids, formed as a result of lipase activity, together with residual triglycerides may stay on the textile during the washing process. Therefore, the quantities of the products left on the swatch appear to be a good measure of the performance of lipases during the washing process. The SLM-test will be disclosed in more detail hereinafter, under the general methods of analysis.

The invention is further illustrated by the following Examples. In the Examples the general methods of analysis were conducted as follows.

Assay for the determination of lipase activity

A. Olive oil hydrolysis (TLU method)

Activities of the lipase preparations of the invention were determined either based on the hydrolysis of olive oil or of p-nitrophenyl-laurate. The former method is essentially according to Näher (G. Näher (1974) in: "Methods of Enzymatic Analysis", Vol. II, pp. 814-818, H.U. Bergmeyer, ed., Academic Press, N.Y., London) except that the fatty acid liberated was measured at pH 8.0 and 25°C in a pH-stat. One True Lipase Unit (TLU) is defined as the titratable fatty acids equivalent to the amount of 1 µmole NaOH per minute.

B. p-Nitrophenyl laurate hydrolysis (NPL method)

The method based on the hydrolysis of p-nitrophenyl laurate to p-nitrophenol and lauric acid is essentially as follows: an appropriate amount of lipase is diluted in 0.05 M MOPS (3-N-Morpholine propane sulfonic aid)-NaOH, pH 8.0 to about 0.003-0.008 Units NPL/ml. One NPL Unit is defined as the amount of lipase necessary to release one µmole of p-nitrophenol. 4 ml of the lipase solution are mixed with 1 ml p-nitrophenyl laurate solution (25 mg p-nitrophenyl laurate in 25 ml ethanol and two drops of 1N HCl) and incubated for 10 min at 30°C. The reaction is stopped by the addition of 5 ml alcoholic-Tris solution (2.5 g Tris-(hydroxymethyl-aminomethane) in 1 l ethanol) and cooled to room temperature. Absorbance is read at 400 nm and corrected for the absorbance of an incubation without lipase. The amount of µmoles p-nitrophenol released is calculated from a calibration curve constructed with the absorbance values at 400 nm of appropriate solutions of p-nitrophenol in ethanol, 1 ml of which is added to 4 ml 0.05 M MOPS-NaOH buffer, pH 8.0, and 5 ml alcoholic-Tris solution.

The stability of the lipases was followed in the time by measuring the activities at 0, 15 and 30 minutes (sometimes up to 90 minutes) with one of these methods.

Assay for the determination of lipase stability in washing solutions

In order to evaluate the stability of a number of lipases according to the invention in washing solutions the following experiments were set up.

A proper amount of a lipase preparation, obtained as described in Example 1, was brought in a solution of:

a) Synthetic Tap Water (STW) containing 0.433 g of $CaCl_2.6aq$, 0.140 g $MgCl_2.6aq$ and 0.210 g $NaHCO_3$ per litre of distilled water,

b) a detergent selected from:

- ALL-base (= ALL without perborate, TAED and enzymes)
- TIDE plus (= TIDE containing sodium tripolyphosphate)
- TIDE minus (phosphate free, containing other builder)

ALL-base was obtained from Unilever Research, The Netherlands. TIDE plus and TIDE minus are commercially available from Procter and Gamble, USA. ALL and TIDE are Registered Trade Marks.

The experiments were carried out at pH 9.0 or 10.3 at 35, 40 or 50°C. Samples were taken from the mixtures at 0, 15 and 30 minutes (sometimes up to 90 minutes), and residual lipase activity was determined with either of the methods described. The lipases used were Pseudomonas pseudoalcaligenes strains Sp 9 (CBS 467.85), IN II-5 (CBS 468.85), Gr VI-15 (CBS 471.85) and M-1 (CBS 473.85), and, for comparative purposes Pseudomonas stutzeri strain Thai IV 17-1 (CBS 461.85) and Acinetobacter calcoaceticus strain Gr V-39 (CBS 460.85).

The results are shown in Examples 4-8.

Assay for the determination of the lipase performance under washing conditions (SLM-test)

The following is a typical Example of how the SLM-test is preferably carried out.

EMPA 211 cotton swatches are used as the fabric and triolein or purified olive oil (both products of Sigma (USA)) as the substrates. The hydrolysis of triolein can be followed by chromatographic methods after extraction of the textile.

The washing procedure preferably employed for the purpose of the SLM-test is as follows:

A volume of 20 µl containing 5 mg olive oil dissolved in acetone (25%) is spotted on a cotton Swatch (3x3 cm). The swatch is air dried at room temperature. The washing solution consisting of 10 ml of STW (Standard Tap Water: 0.433 g $CaCl_2$, 0.140 g $MgCl_2$.6 aq and 0.210 g $NaHCO_3$ per litre of distilled water) or detergent dissolved in STW is placed in an Erlenmeyer flask (25 ml) with a ground stopper and kept in a shaking water-bath at 40 °C. The washing process is started by adding lipase (20 TLU, see hereinafter) and immediately thereafter the soiled swatch, to the Erlenmeyer flask and shaking for 40 min at 40 °C. In a blank experiment no lipase is added.

After washing, the swatch is rinsed with STW and subsequently dried at room temperature. The dried swatches are extracted by rotation in a glass tube containing 5 ml of solvent having the same composition as the eluent used for the chromatographic separation of substrate and products.

In the extraction solution the residual triglyceride and the free fatty acid formed are determined by HPLC.

Equipment and conditions:

Pump :	Model 2150 (LKB)
Detection :	Refractive index monitor (Jobin Jvon).
Injection system :	Wisp (MILLIPORE): 10 µl.
Integrator :	SP 4270 (Spectra Physics)
Column :	CP Microspher-Si (CHROMPACK), 100x4.6 mm.
Eluent :	n-Hexane/isopropylalcohol/formic acid: 975:25:2.5 (v/v), 1 ml/min.
Temperature :	ambient

Under these conditions the retention times of triolein and oleic acid are 1.2 and 1.6 min respectively. The peak area or peak height are measured. They are a measure of the recovery of the triglyceride and fatty acid after extraction from the swatch. The recovery of triglyceride after extraction from the unwashed swatch is taken as 100%.

Under the conditions described above the ratio of the refractive index responses between olive oil and oleic acid was found to be 0.85 on the basis of peak area and 1.1 on the basis of peak height.

Example 1

Preparation of freeze-dried supernatant of a lipase fermentation of strain Sp 9.

Pseudomonas pseudoalcaligenes strain Sp 9 (CBS 467.85) was inoculated in 30 ml sterile brain-heart infusion (BHI) medium (Difco) in a 100 ml conical flask. The culture was shaken for 16 hours at 30 °C in an orbital shaker at 300 rpm. The BHI grown cells were inoculated into a 2 l. conical flask, containing 500 ml of sterile skim milk medium. The skim milk medium was prepared as follows:

- 100 g skim milk (Difco);
- deionized water added to 1 litre;
- the pH is adjusted to 7.0 prior to sterilization;
- sterilization conditions: 110 °C for 30 minutes.

The inoculated skim milk shake flask was shaken for 48 hours in an orbital shaker at 250-300 rpm. The growth temperature for lipase production was 20 °C. The broth was centrifuged with a Sorvall R GSA rotor at 10.000 g. at 8-10 °C. The supernatant obtained after centrifugation was then freeze-dried to give a lipase preparation.

The fermentation of other lipase producing strains, for example strains CBS Nos. 460.85, 461.85, 468.85, 471.85, and 473.85, ATCC No. 29625 and DSM No. 2672 (EP-A-130064) were carried out in a similar way, except that for strain CBS 468.85 the growth temperature was 30 °C.

The lipase preparations obtained according to this Example were used for the stability experiments described in Examples 4-8.

Example 2

Preparation of freeze-dried supernatant of a lipase fermentation of strain Thai IV 17-1.

Pseudomonas stutzeri strain Thai IV 17-1 (CBS 461.85) was inoculated in 30 ml sterile BHI medium in a 100 ml conical flask. The culture was shaken for 16 hours at 30 °C in an orbital shaker at 300 rpm. The BHI grown cells were inoculated into a 2 l conical flask containing 500 ml of sterile GSM medium. The GSM medium was prepared as follows:

- 100 g skim milk (Difco or Oxoid);
- deionized water added to 1 l;
- The pH was adjusted to 7.8 by addition of 4N NaOH;
- The temperature was brought to 40 °C, with stirring;
- MAXATASE® (Gist-brocades) solution was then added;
- The temperature and pH were controlled for 1 hour. Then the pH was brought to 7. Sterilization was carried out at 110 °C for 30 minutes.

The MAXATASE solution was prepared as follows:

To 1 g MAXATASE powder (2.372×10^5 Delft Units/g) deionized water was added to 25 ml. It was shaken well for 5-10 minutes. The insolubles were removed by centrifugation at 10,000 rpm in the SS34 rotor of a Sorvall centrifuge. The supernatant was filtered through a 0.22µ MILLIPORE® filter. The filtrate was then added per litre of 10% skim milk. The inoculated skim milk was shaken in shake flasks for 48 hours at 20 °C in an orbital shaker at 250-300 rpm. Then the broth was centrifuged in a Sorvall GSA rotor at 10,000 g at 8-10 °C. The supernatant obtained was dialysed in EDTA-treated dialysis tubing against 50 volumes of 10mM Tris-HCl buffer, pH 8, at 4 °C, with 2 changes of buffer, over 24 hours. After dialysis the contents of the dialysis sacs were pooled and then freeze-dried.

In a similar way fermentations were carried out with all other strains mentioned in Example 1, except strain CBS 473.85. The lipase containing supernatants obtained according to this Example (except the lipase from strain M-1) were used for the experiments described in Examples 9 and 10.

Example 3

Preparation of freeze-dried supernatant of a lipase fermentation of strain M-1.

Pseudomonas pseudoalcaligenes strain M-1 (CBS 473.85) was inoculated in 500 ml sterile brainheart infusion (BHI) medium (Difco) in a 500 ml conical flask. The culture was shaken for 18 hours at 30 °C in an orbital shaker at 280 rpm. The BHI grown cells were inoculated in a 20 l fermentor containing 10 l of sterile medium 1 or medium 2.

- Medium 1 was prepared as follows:
  Suspend 1000 g skim milk in demineralized water till 9.5 l; bring the temperature at 30 °C and the pH at 7.8 with sodium hydroxide solution; add 33×10^6 ADU (= 26×10^6 DU) MAXACAL^R (Gist-brocades) for protease treatment (30 °C and pH 7.0-7.8) during 1 hour. Autoclavation after adding 0.5-10 ml antifoam (SAG 471 or Pluronic L-81): 30 min. at 110 °C.
- Medium 2 was prepared as follows:
  Suspend 400 g casein in 5 l demineralized water; bring the pH at 9.0 (with a solution of sodium hydroxide) and the temperature at 50 °C; add 5×10^7 ADU (= 4×10^7 DU) Maxacal^R for protease treatment; incubation (90 min. at 50 °C and pH = 9.0) is stopped by fast heating (incubation 5 min at 90 °C). After cooling to 25 °C adjust the pH to 7.0 by sulfuric acid addition.

The other components (butter 50g; $MgSO_4.7aq$ 5 g; $MnSO_4.4aq$ 0.1g and antifoam SAG 471 0.5-10 ml) were added and the medium filled up till 10 l with demineralized water and autoclaved: 65 min. at 120 °C.

The medium was cooled to fermentation temperature (30 °C). Fermentation conditions:

airflow : 0.167 vvm
stirring: 1000 rpm
pH : 6.5 - 10.0 (pref. 6.8-8.0)

The fermentation was continued for between 35-72 hours.

The broth was centrifuged after fermentation on a Hettich centrifuge (4000 g, 8-10 °C) for 30 min. The supernatant was cooled to 0 °C and solid $(NH_4)_2SO_4$ was added to a concentration of 70% (w/w), under stirring. The precipitate formed was separated from the supernatant by centrifugation on a Sorvall centrifuge (10.000 g, 4-5 °C) for 20-25 min. The precipitate was taken up in a 10 mM Tris HCl buffer (pH 8) of 0 °C and cooled acetone (< -7 °C) was added within 20 min. to a concentration of 65% (w/w). After centrifugation the precipitate was taken up in 10 mM Tris HCl buffer pH 8 and dialysed against 50 volumes of the same buffer at 4 °C with 2 changes of buffer over 24 hours. After dialysis the material was lyophilized and the

activity obtained was 6000 TLU/g.

In a similar way all other strains, mentioned in Example 1 can he used, however with the following slight modifications: for strain Sp 9 only medium 1 can be used; the fermentation temperature is 20 °C for all other strains, except for strain IN II-5 which is 20 or 30 °C.

Example 4

Stability experiments with Pseudomonas pseudoalcaligenes lipases at 8 g ALL-base/l at 35, 40 and 50 °C

Source : freeze dried supernatant
Detergent solution: ALL-base, 8 g/l
pH : as indicated

a. Temperature : 35 °C				
Lipase strain	pH	NPL/1 x 10^{-3}	time (min)	residual activity (%)
Sp 9	9	19	0	100
			15	104
			30	106
Sp 9	10.3	19	0	100
			15	88
			30	81
IN II-5	9	11	0	100
			15	107
			30	102
IN II-5	10.3	11	0	100
			15	87
			30	77

b. Temperature : 40 °C					
Lipase strain	pH	TLU/1 or NPL/1 x 10^{-3}		time (min)	residual activity (%)
Sp 9	9.0		14	0	100
				15	102
				30	99
Sp 9	10.3		14	0	100
				15	93
				30	83
IN II-5	9.0		22	0	100
				15	89
				30	79
IN II-5	10.3		22	0	100
				15	76
				30	57
Gr VI-15	10.3	5		0	100
				15	74
				30	61
M 1	10.3	2.8		0	100
				15	63
				30	68

c. Temperature : 50 • C				
Lipase strain	pH	NPL/1 x 10^{-3}	time (min)	residual activity (%)
Sp 9	9.0	14	0 15 30	100 95 80
Sp 9	10.3	14	0 15 30	100 56 32
IN II-5	9.0	22	0 15 30	100 60 49
IN II-5	10.3	22	0 15 30	100 27 12
Gr VI-15	10.3	20.9	0 15 30	100 43 12
M 1	10.3	25	0 15 30	100 62 54

Example 5

Stability experiments with certain Pseudomonas pseudoalcaligenes lipases at 4 g ALL-base/1 at 40 and 50 • C

a.

Source : freeze-dried supernatant
Detergent solution: ALL-base, 4 g/l
pH : as indicated
Temperature : 40 • C

Lipase strain	pH	NPL/1 x 10^{-3}	time (min)	residual activity (%)
Sp 9	9.0	14	0 15 30	100 102 102
Sp 9	10.3	14	0 15 30	100 109 98
IN II-5	9.0	22	0 15 30	100 100 99
IN II-5	10.3	22	0 15 30	100 96 82

b.

Source : Freeze dried supernatant
Detergent solution: ALL-base, 4 g/l
pH : as indicated

Temperature : 50 • C

Lipase strain	pH	NPL/l x 10^{-3}	time (min)	residual activity (%)
Sp 9	9.0	14	0 15 30	100 97 92
Sp 9	10.3	14	0 15 30	100 77 62
IN II-5	9.0	22	0 15 30	100 100 88
IN II-5	10.3	22	0 15 30	100 60 47

Example 6

Stability experiments with Pseudomonas pseudoalcaligenes strain Sp 9 at 6 g TIDE/1 at 40 and 50 • C

Source : freeze-dried supernatant of Sp 9
Detergent solution: as indicated, 6 g/l
pH : as indicated

a. Temperature : 40 • C				
Detergent	pH	NPL/1 x 10^{-3}	time (min)	residual activity (%)
TIDE minus	9.0	14	0 15 30	100 118 115
TIDE minus	10.3	14	0 15 30	100 106 107
TIDE plus	9.0	14	0 15 30	100 129 127
TIDE plus	10.3	14	0 15 30	100 118 105

b. Temperature : 50 • C				
Detergent	pH	NPL/1 x 10^{-3}	time (min)	residual activity (%)
TIDE minus	9.0	14	0	100
			15	107
			30	107
TIDE minus	10.3	14	0	100
			15	79
			30	67
TIDE plus	9.0	14	0	100
			15	120
			30	127
TIDE plus	10.3	14	0	100
			15	81
			30	54

Example 7

Stability experiments of Pseudomonas stutzeri lipase Thai IV 17-1 at 5 g ALL-base/1 at 40 • C

Source : freeze dried supernatant of Thai IV 17-1
Detergent solution: ALL-base, 5 g/l
pH : 9.0
Temperature : 40 • C

Time (min)	resid. act (%)
0	100
25	109

Example 8

Stability experiments with Acinetobacter calcoaceticus lipase at 8 g ALL-base/1 at 40 and 50 • C

Source : freeze-dried supernatant of Gr V-39 (CBS 460.85)
Detergent solution : ALL-base, 8 g/l
pH : 10.3

a. Temperature : 40 • C		
TLU/1 x 10^{-3}	time (min)	residual activity (%)
4.9	0	100
	15	100
	30	80

b. Temperature : 50 °C		
NPL/1 x 10^{-3}	time (min)	residual activity (%)
23.6	0	100
	15	27
	30	5

Example 9

This Example illustrates the performance of lipase strains Ps. pseudoalcaligenes Sp 9, IN II-5, Gr VI-15, M-1. ATCC 29625, Ps. stutzeri Thai II-5 and Acetobacter calcoaceticus Gr V-39 in a washing process according to the SLM-test, where the compatibility of these enzymes with a builder component present in modern washing compositions (TPP), a powder detergent composition (ALL-base) and a liquid formulation (TIDE liquid) was checked.

The SLM-test was carried out as described on pages 18-19 of this specification. Preliminary experiments showed that, under the conditions used, a considerable amount of fatty acids formed by lipase, together with residual triglyceride stayed on the textile during the washing process.

The performance of the lipases mentioned above and the blank was tested by the SLM-method under the following conditions:

- standard tap water (STW) adjusted to pH 9.1 with alkali.
- sodium tripolyphosphate (TPP): 2 and 10 g/l (pH 9.1).
- liquid TIDE : 2 and 4 g/l (pH 7.5)
- ALL-base : 2, 4 and 8 g/l (pH 9.2-9.6)

The lipase activity units added (20 TLU) were obtained from the freeze-dried samples produced according to Example 2 and in case of strain M-1 to Example 3. They were found to be as follows:

Strain	Sp 9	IN II-5	Gr VI-15	M-1	ATCC 29625
Activ. (TLU/g)	70	130	50	6000	35

Strain	Thai IV 17-1	Gr V-39
Activ. (TLU/g)	165	62.5

The results are shown in the following tables.

A. Alkaline lipase: none (blank)		
Condition	(g/l)	Recovery (%)
		Triglycerides
STW	-	92.7
TPP	2	80.6
TPP	10	85.0
Liquid TIDE	2	63.5
Liquid TIDE	4	63.9
ALL-base	2	65.3
ALL-base	4	65.8
ALL-base	8	63.4

B. Alkaline lipase from strain Sp 9				
Condition	(g/l)	Recovery (%)		
		Triglycerides	Free fatty acids	Total
STW	-	9.4	91.0	100.4
TPP	2	32.3	45.4	77.7
TPP	10	34.4	19.3	53.7
Liquid TIDE	2	23.1	25.3	48.4
Liquid TIDE	4	29.4	20.2	49.6
ALL base	2	24.1	30.4	54.5
ALL base	4	34.6	21.3	55.9

C. Alkaline lipase from strain IN II-5				
Condition	(g/l)	Recovery (%)		
		Triglycerides	Free fatty acids	Total
STW	-	20.4	68.3	88.6
TPP	2	11.6	61.0	72.6
TPP	10	39.3	6.3	45.6
Liquid TIDE	2	35.6	16.0	51.6
Liquid TIDE	4	37.8	13.3	51.1
ALL base	2	6.1	66.4	72.6
ALL base	4	20.6	30.6	51.2
ALL base	8	44.1	11.0	55.2

D. Alkaline lipase from strain M-1				
Condition	(g/l)	Recovery (%)		
		Triglycerides	Free fatty acids	Total
STW	-	9.3	75.9	85.2
TPP	2	24.7	38.8	63.5
TPP	10	32.8	9.9	42.7
Liquid TIDE	2	33.3	12.0	45.3
Liquid TIDE	4	37.5	16.4	53.9
ALL-base	2	12.6	37.3	49.9
ALL-base	4	29.1	15.7	44.8

E. Alkaline lipase from strain Gr VI-15				
Condition	(g/l)	Recovery (%)		
		Triglycerides	Free fatty acids	Total
STW	-	22.0	59.3	81.3
TPP	2	24.1	48.5	72.6
TPP	10	31.5	28.1	59.6
Liquid TIDE	2	49.3	9.5	58.8
Liquid TIDE	4	54.4	0.0	54.4
ALL-base	2	32.0	23.0	55.0
ALL-base	4	31.3	15.6	52.9

F. Alkaline lipase from strain ATCC 29625 (Citrulli)				
Condition	(g/l)	Recovery (%)		
		Triglycerides	Free fatty acids	Total
STW	-	2.8	102.5	105.3
TPP	2	3.8	89.0	92.9
TPP	10	42.9	13.0	55.9
Liquid TIDE	2	10.9	54.9	65.8
Liquid TIDE	4	19.2	27.2	46.4
ALL-base	2	24.7	40.5	65.2
ALL-base	4	36.2	20.0	56.2

G. Alkaline lipase from Thai IV-17-1				
Condition	(g/l)	Recovery (%)		
		Triglycerides	Free fatty acids	Total
STW	-	4.3	75.3	79.6
TPP	2	24.7	29.9	54.7
TPP	10	44.6	10.1	54.8
Liquid TIDE	2	21.2	29.3	50.5
Liquid TIDE	4	24.6	17.9	42.6
ALL-base	2	23.9	27.2	51.2
ALL-base	4	47.0	10.0	57.0

H. Alkaline lipase from strain Gr V-39				
Condition	(g/l)	Recovery (%)		
		Triglycerides	Free fatty acids	Total
STW	-	6.2	91.6	97.9
TPP	2	15.5	53.3	68.8
TPP	10	57.4	9.3	66.7
ALL-base	2	28.6	16.8	45.3
ALL-base	4	25.7	14.4	40.1
Liquid TIDE	2	33.7	13.2	46.9

From these tables it clearly appears that the lipases of the present invention show their lipolytic properties on textile, and, in particular, their excellent performance in liquid and powder detergents under washing conditions.

Example 10

This Example shows the compatibility of certain lipases of the present invention and bleach or a protein splitting enzyme under washing conditions. The performance of the lipases was tested by the SLM-method, as described on pages 18-19 of this specification under the following conditions:

- ALL-base + bleach activator (TAED 3%): 4 g/l (pH 9.1).
- ALL-base + TAED + bleach ($NaBO_3$.4aq., 13%) : 4 g/l (pH 9.1)
- ALL-base + TAED + protease (2000 DU/g detergent): 4 g/l (pH 9.1)

The lipase activity units added (20 TLU) were obtained from the freeze-dried samples produced according to Example 2 and in the case of M1 to Example 3 (see Example 9).
Lipase strains : IN II-5, M1, Gr VI-15 and ATCC 29625.
Protease : MAXATASE. The proteolytic activity of MAXATASE (DU/1) was determined according to the Delft Method, described in J. Amer. Oil Chem Soc. 60 (1983), 1672.
The results, expressed in the same way as in Example 9, are shown in the following tables:

A. Alkaline lipase from strain IN II-5			
Condition	Recovery (%)		
	Triglyceride	Free fatty acid	Total
ALL-base + TAED	4.4	62.4	66.8
ALL-base + TAED + bleach	5.7	64.5	70.2
ALL-base + TAED + MAXATASE	2.2	61.1	63.3

B. Alkaline lipase from strain M-1			
Condition	Recovery (%)		
	Triglyceride	Free fatty acid	Total
ALL-base + TAED	35.1	16.1	51.2
ALL-base + TAED + bleach	38.1	14.9	53.0
ALL-base + TAED + MAXATASE	41.5	10.0	51.6

C. Alkaline lipase from strain GrVI-15			
Condition	Recovery (%)		
	Triglyceride	Free fatty acid	Total
ALL-base + TAED	42.0	7.4	49.4
ALL-base + TAED + bleach	43.2	7.5	50.7
ALL-base + TAED + MAXATASE	42.3	4.5	46.9

D. Alkaline lipase from strain ATCC 29625			
Condition	Recovery (%)		
	Triglyceride	Free fatty acid	Total
ALL-base + TAED	34.7	19.8	54.5
ALL-base + TAED + bleach	32.6	27.5	60.1
ALL-base + TAED + MAXATASE	36.4	22.4	58.8

E. Alkaline lipase from strain Thai IV 17-1			
Condition	Recovery (%)		
	Triglyceride	Free fatty acid	Total
ALL-base + TAED	31.0	17.2	48.2
ALL-base + TAED + bleach	32.9	13.8	46.7
ALL-base + TAED + MAXATASE	32.5	16.1	48.6

Also from these tables it clearly appears that the lipases of the present invention show their lipolytic properties on textile, and, in particular, their excellent performance in liquid and powder detergents under washing conditions.

Claims

1. An enzymatic detergent additive, the active component of which is a lipolytic enzyme, characterized in that the lipolytic enzyme is an enzyme obtained from a lipase producing strain selected from the group consisting of Pseudomonas pseudoalcaligenes CBS 467.85, CBS 468.85, CBS 471.85, CBS 473.85 and ATCC 29625, and mutants and variants thereof, said enzyme being further characterized by:
   a) a pH optimum in the range of 8 to 10.5, measured in a pH-stat under conditions of TLU determination;
   b) exhibiting effective lipolytic activity in an aqueous solution containing a detergent at a concentration up to 10 g/l of solution under washing conditions at a temperature of 60 °C or below and at a pH between 7 and 11.

2. An enzymatic detergent additive according to Claim 1, characterized in that it further comprises a proteolytic enzyme and, optionally, an amylolytic enzyme.

3. An enzymatic detergent additive according to Claim 1 or 2, characterized in that the lipase exhibits lipolytic activity in the range of 10^2 to 10^6 TLU/g of additive.

4. An enzymatic detergent additive according to Claim 2 or 3, characterized in that the protease exhibits proteolytic activity of $5x10^4$ to 10^5 DU/g of additive.

5. An enzymatic detergent additive according to any one of Claims 1 to 4, characterized in that it is in the form of a granulate or prill.

6. An enzymatic detergent additive according to any one of Claims 1 to 4, characterized in that it is in the form of a liquid with an enzyme stabilizer.

7. A detergent composition, characterized in that it comprises an enzymatic detergent additive according to any one of Claims 1 to 6, together with a surfactant and, optionally, other ingredients commonly used in such compositions.

8. A detergent composition according to Claim 7, characterized in that it has lipolytic activity in the range of 1 to 20,000 TLU/g of composition.

9. A detergent composition according to Claim 7 or 8, characterized that it comprises a proteolytic enzyme in addition to the lipolytic enzyme, which has proteolytic activity in the range of 50 to 10,000 Delft Units/g of composition.

10. A washing process, characterized in that it comprises the use of a detergent composition as claimed in any one of Claims 7 to 9 wherein the pH during the washing is in the range of 7 to 11 and the temperature is 60 °C or lower.

11. A process for producing a lipolytic enzyme suitable for use in detergents, characterized in that a lipase producing strain of Pseudomonas pseudoalcaligenes is cultivated in a nutrient medium therefor to form a lipase rich broth and lipase is isolated from the broth, with the proviso that the Pseudomonas pseudoalcaligenes strain is not ATCC 29625 nor ATCC 33996.

12. A process according to Claim 11, characterized in that the Pseudomonas pseudoalcaligenes strain is CBS 467.85, CBS 468.85, CBS 471.85 or CBS 473.85.

13. Pseudomonas pseudoalcaligenes Sp9, having the deposit accession number CBS 467.85.

14. Pseudomonas pseudoalcaligenes IN II-5, having the deposit accession number CBS 468.85.

15. Pseudomonas pseudoalcaligenes GR VI-15, having the deposit accession number CBS 471.85.

16. Pseudomonas pseudoalcaligenes M-1, having the deposit accession number CBS 473.85.

17. Use of a lipolytic enzyme from a lipase producing strain of Pseudomonas pseudoalcaligenes, as an active component in a detergent composition or an enzymatic detergent additive.

Revendications

1. Additif enzymatique pour détergent dont le composant actif est une enzyme lipolytique, caractérisé en ce que l'enzyme lipolytique est une enzyme obtenue à partir d'une souche produisant de la lipase choisie dans la classe formée par Pseudomonas pseudoalcaligenes CBS 467.85, CBS 468.85, CBS 471.85, CBS 473.85 et ATCC 29625 et leurs mutants et variants, laquelle enzyme est caractérisée de surcroît :
   a) par un pH optimum de l'intervalle de 8 à 10,5, mesuré dans un pH-stat dans les conditions de détermination de l'unité de lipase vraie;
   b) par la manifestation d'une activité lipolytique effective dans une solution aqueuse contenant un détergent à une concentration s'élevant jusqu'à 10 g par litre de solution dans les conditions du lavage à une température de 60 °C sinon moins et à un pH entre 7 et 11.

2. Additif enzymatique pour détergent suivant la revendication 1, caractérisé en ce qu'il comprend, en outre, une enzyme protéolytique et facultativement une enzyme amylolytique.

3. Additif enzymatique pour détergent suivant la revendication 1 ou 2, caractérisé en ce que la lipase manifeste une activité lipolytique de l'intervalle de 10^2 à 10^5 unités de lipase vraie par g d'additif.

4. Additif enzymatique pour détergent suivant la revendication 2 ou 3, caractérisé en ce que la protéase manifeste une activité protéolytique de 5×10^4 à 10^6 unités Delft par g.

5. Additif enzymatique pour détergent suivant l'une quelconque des revendications 1 à 4, caractérisé en ce qu'il se présente sous la forme de granules ou de grenaille.

6. Additif enzymatique pour détergent suivant l'une quelconque des revendications 1 à 4, caractérisé en ce qu'il se présente sous la forme d'un liquide avec un stabilisant d'enzyme.

7. Composition détergente, caractérisée en ce qu'elle comprend un additif enzymatique pour détergent suivant l'une quelconque des revendications 1 à 6, conjointement avec un tensioactif et facultativement d'autres constituants d'usage courant dans de telles compositions.

8. Composition détergente suivant la revendication 7, caractérisée en ce qu'elle a une activité lipolytique de l'intervalle de 1 à 20000 unités de lipase vraie par g de composition.

9. Composition détergente suivant la revendication 7 ou 8, caractérisée en ce qu'elle comprend une enzyme protéolytique en plus de l'enzyme lipolytique, laquelle a une activité protéolytique de l'intervalle de 50 à 10000 unités Delft par g de composition.

10. Procédé de lavage, caractérisé en ce qu'il comprend l'utilisation d'une composition détergente suivant l'une quelconque des revendications 7 à 9 où le pH pendant le lavage se situe dans l'intervalle de 7 à 11 et la température est de 60 °C sinon moins.

11. Procédé de production d'une enzyme lypolytique se prêtant à l'utilisation dans les détergents, caractérisé en ce qu'on cultive une souche produisant de la lipase de Pseudomonas pseudoalcaligenes dans un milieu nutritif pour celle-ci pour former un bouillon riche en lipase et on isole la lipase du bouillon, avec la restriction que la souche de Pseudomonas pseudoalcaligenes n'est pas ATCC 29625 ni ATCC 33996.

12. Procédé suivant la revendication 11, caractérisé en ce que la souche de Pseudomonas pseudoalcaligenes est CBS 467.85, CBS 468.85, CBS 471.85 ou CBS 473.85.

13. Pseudomonas pseudoalcaligenes Sp9, ayant le numéro d'accès au dépôt CBS 467.85.

14. Pseudomonas pseudoalcaligenes IN II-5, ayant le numéro d'accès au dépôt CBS 468.85.

15. Pseudomonas pseudoalcaligenes GR VI-15, ayant le numéro d'accès au dépôt CBS 471.85.

16. Pseudomonas pseudoalcaligenes M-1, ayant le numéro d'accès au dépôt CBS 473.85.

17. Utilisation d'une enzyme protéolytique provenant d'une souche de Pseudomonas pseudoalcaligenes produisant de la lipase comme composant actif dans une composition détergente ou un additif enzymatique pour détergent.

Patentansprüche

1. Enzymatisches Waschmitteladditiv, dessen aktive Komponente ein lipolytisches Enzym ist, dadurch gekennzeichnet, dass das lipolytische Enzym ein Enzym ist, das von einem Lipase erzeugenden Stamm erhalten wird, der aus der Gruppe gewählt ist, die aus Pseudomonas pseudoalcaligenes CBS 467.85, CBS 468.85, CBS 471.85, CBS 473.85 und ATCC 29 625 und Mutanten und Varianten davon besteht, wobei das Enzym ferner gekennzeichnet ist durch
   a) ein pH-Optimum im Bereich von 8 bis 10,5, gemessen in einem pH-Stat unter Bedingungen der TLU-Bestimmung;
   b) Aufweisen von wirksamer lipolytischer Aktivität in einer wässrigen Lösung, die ein Waschmittel bei einer Konzentration von bis zu 10 g/l Lösung enthält, unter Waschbedingungen bei einer Temperatur von 60 °C oder darunter und bei einem pH zwischen 7 und 11.

2. Enzymatisches Waschmitteladditiv nach Anspruch 1, dadurch gekennzeichnet, dass es ferner ein proteolytisches Enzym und gegebenenfalls ein amylolytisches Enzym enthält.

3. Enzymatisches Waschmitteladditiv nach Anspruch 1 oder 2, dadurch gekennzeichnet, dass die Lipase lipolytische Aktivität im Bereich von 10^2 bis 10^6 TLU/g Additiv aufweist.

4. Enzymatisches Waschmitteladditiv nach Anspruch 2 oder 3, dadurch gekennzeichnet, dass die Protease proteolytische Aktivität von 5×10^4 bis 10^5 DU/g Additiv aufweist.

5. Enzymatisches Waschmitteladditiv nach einem der Ansprüche 1 bis 4, dadurch gekennzeichnet, dass es in Form eines Granulats oder Prills vorliegt.

6. Enzymatisches Waschmitteladditiv nach einem der Ansprüche 1 bis 4, dadurch gekennzeichnet, dass es in Form einer Flüssigkeit mit einem Enzymstabilisator vorliegt.

7. Waschmittelformulierung, dadurch gekennzeichnet, dass sie ein enzymatisches Waschmitteladditiv nach einem der Ansprüche 1 bis 6 zusammen mit einem oberflächenaktiven Mittel und gegebenenfalls anderen Bestandteilen, die gewöhnlich in solchen Formulierungen verwendet werden, aufweist.

8. Waschmittelformulierung nach Anspruch 7, dadurch gekennzeichnet, dass sie eine lipolytische Aktivität im Bereich von 1 bis 20 000 TLU/g Formulierung hat.

9. Waschmittelformulierung nach Anspruch 7 oder 8, dadurch gekennzeichnet, dass sie zusätzlich zu dem lipolytischen Enzym ein proteolytisches Enzym aufweist, das eine proteolytische Aktivität im Bereich von 50 bis 10 000 Delft-Einheiten/g Formulierung hat.

10. Waschverfahren, dadurch gekennzeichnet, dass es die Verwendung einer Waschmittelformulierung nach einem der Ansprüche 7 bis 9 umfasst, wobei der pH-Wert während des Waschens im Bereich von 7 bis 11 liegt und die Temperatur 60 °C oder niedriger ist.

11. Verfahren zur Herstellung eines lipolytischen Enzyms, das für die Verwendung in Waschmitteln geeignet ist, dadurch gekennzeichnet, dass ein Lipase erzeugender Stamm von Pseudomonas pseudoalcaligenes in einem Nährmedium dafür kultiviert wird, um eine lipasereiche Kulturflüssigkeit zu bilden, und Lipase aus der Kulturflüssigkeit isoliert wird, mit der Massgabe, dass der Pseudomonas pseudoalcaligenes-Stamm weder ATCC 29 625 noch ATCC 33 996 ist.

12. Verfahren nach Anspruch 11, dadurch gekennzeichnet, dass der Pseudomonas pseudoalcaligenes-Stamm CBS 467.85, CBS 468.85, CBS 471.85 oder CBS 473.85 ist.

13. Pseudomonas pseudoalcaligenes Sp9 mit der Hinterlegungsordnungsnummer CBS 467.85.

14. Pseudomonas pseudoalcaligenes IN II-5 mit der Hinterlegungsordnungsnummer CBS 468.85.

15. Pseudomonas pseudoalcaligenes GR VI-15 mit der Hinterlegungsordnungsnummer CBS 471.85.

16. Pseudomonas pseudoalcaligenes M-1 mit der Hinterlegungsordnungsnummer CBS 473.85.

17. Verwendung eines lipolytischen Enzyms von einem Lipase erzeugenden Stamm von Pseudomonas pseudoalcaligenes als aktive Komponente in einer Waschmittelformulierung oder einem enzymatischen Waschmitteladditiv.